BEHAVIOR OF THE NURSE-CELLS OF LITTORINA IRRORATA (SAY)

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As the writer has shown (in press) the mode of development of the nurse-cells of *Littorina irrorata* is in many respects such as to indicate their homology to the atypical spermatozoa of other prosobranchs. The present paper is concerned with the behavior and history of the nurse-cells since they furnish additional support to this view.

The methods used consisted simply in the examination of temporary mounts and permanent smears of the contents of the different parts of the reproductive tract of both sexes. This material was secured with a pipette during dissection of the animals in sea water. The smears were fixed and stained by the usual methods. These were useful as a check upon the observations made on living material, for photographs and as a permanent record of the observations.

Behavior of the Nurse-cells and Spermatozoa from the Sperm Duct

The general appearance of the nurse-cells with tufts of quiescent spermatozoa attached is shown in Figure 1. Such nurse cells were found abundantly in the first (coiled) portion of the sperm duct of every male examined (July). The seminal vesicle, prostate and the distal portions of the sperm duct were entirely empty in every case. When the nurse-cells are removed from the duct and examined in sea water all of the spermatozoa are at first quiescent. Within a few minutes, however, some of them begin to show slight movements of their tails. These movements gradually increase in amplitude, and more and more of the spermatozoa begin to move in the same way until the entire group attached to a given nurse-cell is beating rhythmically. The nurse-cell is thus propelled through the water until it reaches some obstacle or until the tails of the sperm of one tuft become entangled with those of another. The latter occurs in more and more tufts the longer the cul-

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ture is maintained until finally (in about 30 minutes usually) hundreds of individual tufts have become entangled with each other. The nurse-cells themselves lie peripherally and are moved passively to and fro with the beating movements of the tufts. The three-dimensional pinwheels formed in the above manner (Fig. 4) often are seen to revolve in the liquid medium, if space permits. They may undergo progressive movement if there happen to be more sperm tufts on one side than on the other. The formation of these aggregates gives the culture a spotted appearance which is visible to the naked eye.

After some time a few of the spermatozoa of each aggregate become detached from the nurse-cells. Other spermatozoa become detached until finally all of the sperm attached to a given nurse-cell have lost contact with it. When this happens the nurse-cell set free in this way is forced peripherally by the currents set up by the moving spermatozoa. When all of the nurse-cells are free the spermatozoa still form an aggregate being completely entangled by their tails. These latter aggregates persist for some time. The heads of the spermatozoa are radially disposed around the periphery of the aggregate. After a time the spermatozoa become disentangled from the aggregate and swim freely away from it. The free spermatozoa show a marked thigmotaxis and soon become attached by their heads to any available surface but not to the free nurse-cells.

Behavior of Nurse-cells in Female

Some 200 females were dissected in order to follow the history and fate of the nurse-cells and spermatozoa. In only a small percentage of cases was a recent copulate to be found. The great majority of the females were found with empty uteri, but nearly all of them contained a large number of spermatozoa in the seminal receptacle. This organ is

PLATE 1

FIG. 1. Smear from sperm duct showing nurse-cells with attached spermatozoa. Osmic vapor. × 579.

FIG. 2. Smear from uterus showing nurse-cells with contained spermatozoa. A few free spermatozoa are to be seen here and there. Zenker's fluid without acetic. × 579.

FIG. 3. Proximal end of spermatophore taken from uterus showing the spermatozoa agglutinated head-to-head after segregation from nurse-cells. Osmic vapor. × 48.

FIG. 4. Three-dimensional pinwheel aggregate formed of many nurse-cells with attached sperm tufts. Somewhat distorted prior to fixation. Osmic vapor. × 48.

FIG. 5. Distal end of same spermatophore shown in Figure 3 showing the nurse-cells segregated from spermatozoa and about to be extruded. × 48.

FIG. 6. Section through female at the level of the seminal receptacle showing typical spermatozoa attached by their heads to the lining cells. × 5.6.

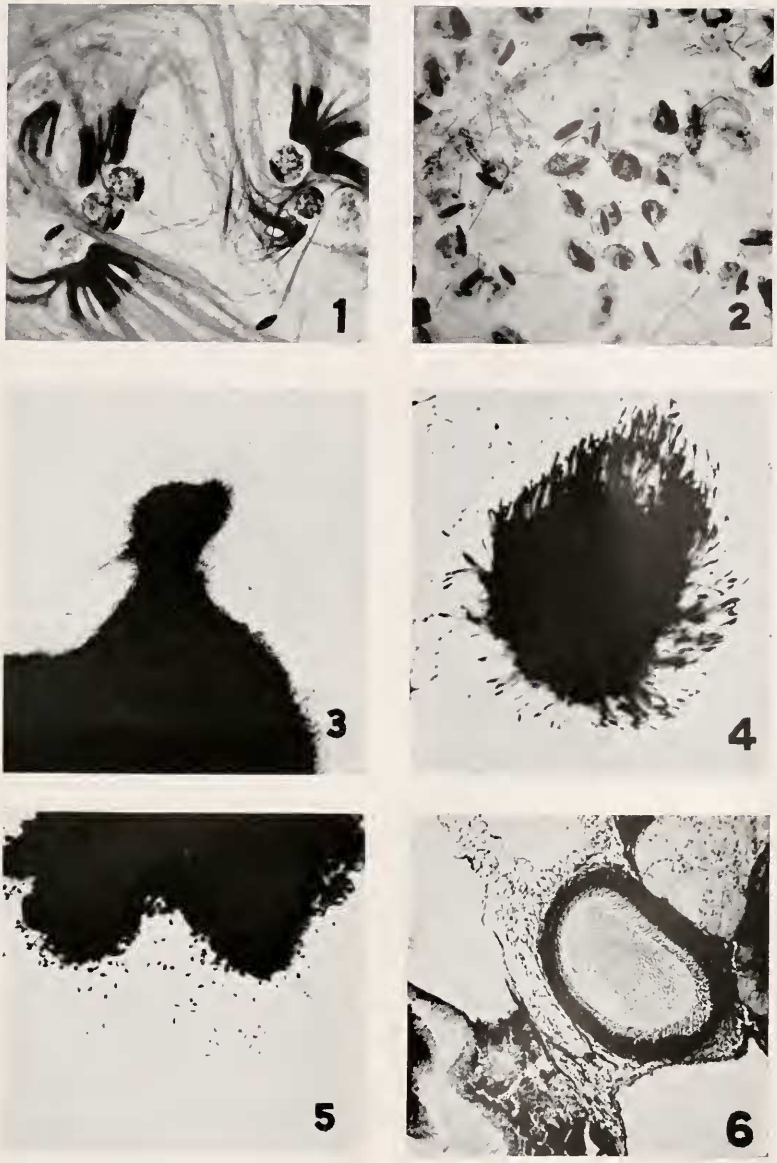

PLATE I

Figures 1 and 2 were made with a 4 mm., apochromatic objective (N.A. 0.95), Figures 3, 4 and 5 with a 16 mm., apochromatic objective (N.A. 0.30), and Figure 6 with a 32 mm., microtessar. The magnification of each figure is given in the descriptive legend.

a blindly ending sac lying posterior to the site of sperm deposition. Figure 6 shows a section through a female at the level of the seminal receptacle. The heads of the spermatozoa are attached to the epithelial cells lining the receptacle, the tails projecting into the lumen. No nurse-cells were found in the seminal receptacle. Linke (1933) found the same arrangement in the species which he studied, and he also observed sections of *L. obtusata* which included an egg as it passed the opening of the seminal receptacle into the oviduct. The egg was surrounded by spermatozoa which appeared to be streaming from the seminal receptacle, indicating that this is the site of fertilization.

In three of the females examined, the distal end of the uterus contained a cylindrical mass of semen which showed considerable resistance to breakdown when removed for study. In one case this mass contained a large number of nurse-cells with attached spermatozoa, in another case it consisted of nurse-cells and free spermatozoa, and in a third of nurse-cells only. Many of the nurse-cells in the latter copulate were swollen or otherwise distorted. In the second case cited above the nurse-cells were located at the distal end of the mass only, the spermatozoa being segregated at the opposite or proximal end, i.e., nearer the seminal receptacle (see Figs. 5 and 3, respectively).

In two of the females in which the above described sperm mass was not present the uterus contained, instead, a large number of swollen nurse-cells and a few spermatozoa. The nurse-cells were in the act of ingesting living spermatozoa, and many of them contained hundreds of sperm (Fig. 2). In many cases spermatozoa could be seen within these cells, coiling and writhing about in a large watery vacuole contained in the nurse-cell.

The spermatozoa of *L. irrorata* show a pronounced head-to-head agglutination reaction when not attached to nurse-cells. This is true of segregated sperm taken from the seminal receptacle as well as of those taken from the proximal end of a recent copulate. This agglutination can be seen in Figure 3 which represents the proximal end of such a copulate.

Discussion

The most remarkable aspect of the history of the nurse-cells lies in their behavior when withdrawn from the sperm duct. The formation of the three-dimensional pinwheels is similar in many respects to the formation of clumps of apyrene and eupyrene spermatozoa described by the writer (Woodard, 1940) for *Goniobasis:* 1. Both are brought about by the sticking together or entanglement of spermatozoa by their tails. 2. Both result in the formation of temporary aggregates. 3. In both

cases the atypical components play out their role and from this time on show no further ordered behavior towards the spermatozoa. 4. In *Goniobasis* a tail-to-tail aggregation of the spermatozoa is a condition intermediate to their final disposition (in the seminal receptacle). This is presumably also the case in *L. irrorata.* 5. In both cases these aggregates eventually disperse, and the free spermatozoa show a tendency to wander away from the site of aggregation, and a marked thigmotaxis. 6. In both cases the spermatozoa eventually become sharply segregated from the atypical structures and reach a seminal receptacle where they are stored. 7. In both cases the atypical structures undergo breakdown and extrusion after their functional phase has passed. 8. Finally, in both cases the atypical structures act as an aid in the aggregation. In the case of *Goniobasis* as I have shown experimentally, the atypical spermatozoa are necessary. In the case of *L. irrorata,* it may be emphasized, both nurse-cells and spermatozoa may be present separately, but when not attached the spermatozoa aggregate head-to-head (agglutinate) rather than by their tails. This would seem to indicate that an atypical structure is necessary for tail-to-tail aggregation.

The observations of Tyler (1940) may be of great significance in this connection. He has shown that in *Megathura crenulata* two types of sperm aggregation occur: one is a head-to-head aggregation which he regards as primarily a dilution effect; the other is a tail-to-tail agglutination which is effected by a substance present in the jelly layer of the egg. The existence of such a specific tail-to-tail agglutinin suggests that, in the case of both the apyrene spermatozoa of *Goniobasis* and the nurse-cells of *Littorina,* we may be dealing with structures which can in some way produce the same effect upon the spermatozoa as the substance from the jelly layer of the egg in Tyler's experiments. It may thus be possible to arrive at a biochemical proof of the essentially egg-like character of the atypical spermatozoa of prosobranchs where the morphological proof remains more or less indecisive.

Summary

The nurse-cells of *L. irrorata* with tufts of sperm attached are moulded in the male into rudimentary spermatophores and are then passed to the female. In the uterus the sperm become detached from the nurse-cells and migrate to the seminal receptacle where they are stored. The nurse-cells then ingest any remaining spermatozoa, break them down, and are themselves eventually extruded. Nurse-cells with tufts of sperm in the sperm duct show an aggregation reaction which leads to the formation of three-dimensional pinwheels. The tufts of

sperm are attached by their tails to each other, and the nurse-cells lie radially disposed. These aggregates disperse first by detachment of the nurse-cells, leaving the sperm attached by their tails, then by disentanglement of each of the sperm. The free sperm then show a thigmotaxis. Sperm not attached to nurse-cells (from seminal receptacle or from a copulate in which segregation has already occurred) show the usual head-to-head agglutination reaction. The behavior of the nurse-cells and spermatozoa is viewed as a mechanism whereby the latter are segregated from the former. This behavior is compared with the clumping of the apyrene and eupyrene spermatozoa of *Goniobasis*.

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